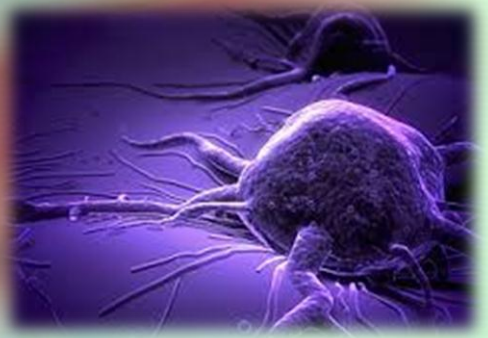
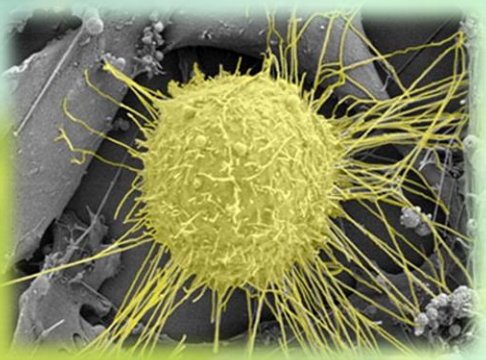
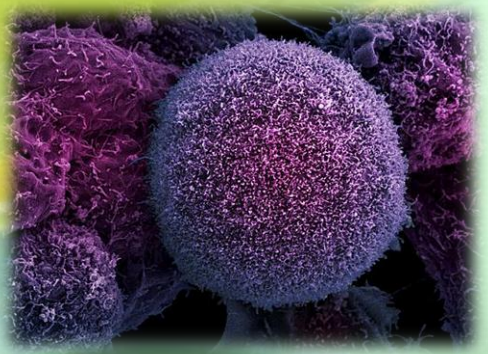
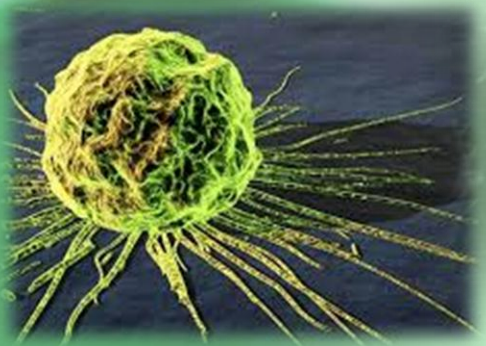





In the Name of God

Qazvin University of Medical Science
Department of Immunology & Microbiology

Journal Club & MSc Seminar

Turning Anthrax Toxin into A Cancer Killer

Presented by: Mohammad Sekhavati

Supervised by: Dr.M.Aslanimehr

Background

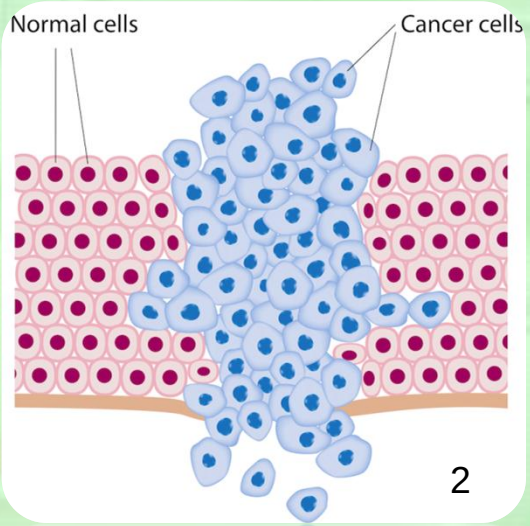
Cancer is characterized by uncontrolled and invasive growth of cells (metastasis)

- The second ranking cause of death in the industrialized world

Conventional anticancer therapies

- surgical resection, radiotherapy and chemotherapy
- ineffective (about half of cancer sufferers)
- alternative techniques are being developed

Experimental cancer treatments



Experimental cancer treatments

Improving, supplementing or replacing conventional methods

- Photodynamic therapy
- HAMLET (human alpha lactalbumin made lethal to tumor cells)
- Gene therapy
- Telomerase therapy
- Hyperthermia therapy
- Dichloroacetate (DCA)
- Diet therapy
- Insulin potentiating therapy
- **Bacterial treatment**

Many of these therapies are controversial due to lack of evidence, efficacy, feasibility, availability, specificity and selectivity

Bacterial therapy

The first scientifically-observed treatment dates back to the Nineteenth century

- *Busch* (1868)
  - Certain types of cancers regressed following accidental ***erysipelas*** (*Streptococcus pyogenes)* infections
- *William Coley* (1991)
  - The patient with neck cancer began to recover following an infection with ***erysipelas***
  - Coley's toxins (a safer vaccine)
  - a mixture of inactivated ***S. pyogenes*** and ***Serratia marcescens***
  - sarcomas, carcinomas, lymphomas, melanomas and myelomas
- Certain species of anaerobic bacteria (the genus *Clostridium*)
  - Thrive and consume oxygen-poor cancerous tissue

Bacterial therapy

1. Bacteria as **tumoricidal** agents
2. Bacteria as **vector** for gene therapy
3. Bacterial **toxins** for cancer treatment
4. Bacteria as **immunotherapeutic** agents
5. Bacterial **spores**

Advantageous features such as capacity to simultaneously carry and express multiple therapeutic proteins, and elimination by antibiotics

Bacteria as tumoricidal agents

live, attenuated (genetically-modified), nonpathogenic bacteria

1. To provide direct tumoricidal effects
2. To deliver tumoricidal molecules

The potential to target and colonize solid tumors could be shown for many different bacteria

- Obligate anaerobic bacteria like *Clostridia* or *Bifidobacteria*
- Facultative anaerobic bacteria like *Salmonellae* or *E. coli*

(The **hypoxic conditions** that are found in necrotic areas of solid tumors)

✓ The major problem with using bacteria as anti-cancer agents is their toxicity at the dose required for therapeutic efficacy

✓ Restrict to preclinical animal models

Live bacterial anti-tumor agents

Bacillus Calmette-Guerin (BCG)

- A derivative of *Mycobacterium bovis* (vaccine strain against tuberculosis)
- In 1950s BCG was studied as an anticancer agent
- BCG plus TURBT the most successful treatment
- Immunotherapy: induces complex immune response
- Dramatically decreases the recurrence rates in 70% of the patients
- Instillation of BCG directly to tumor site (2-3 weeks after surgery)
  - Once a week for 6 weeks

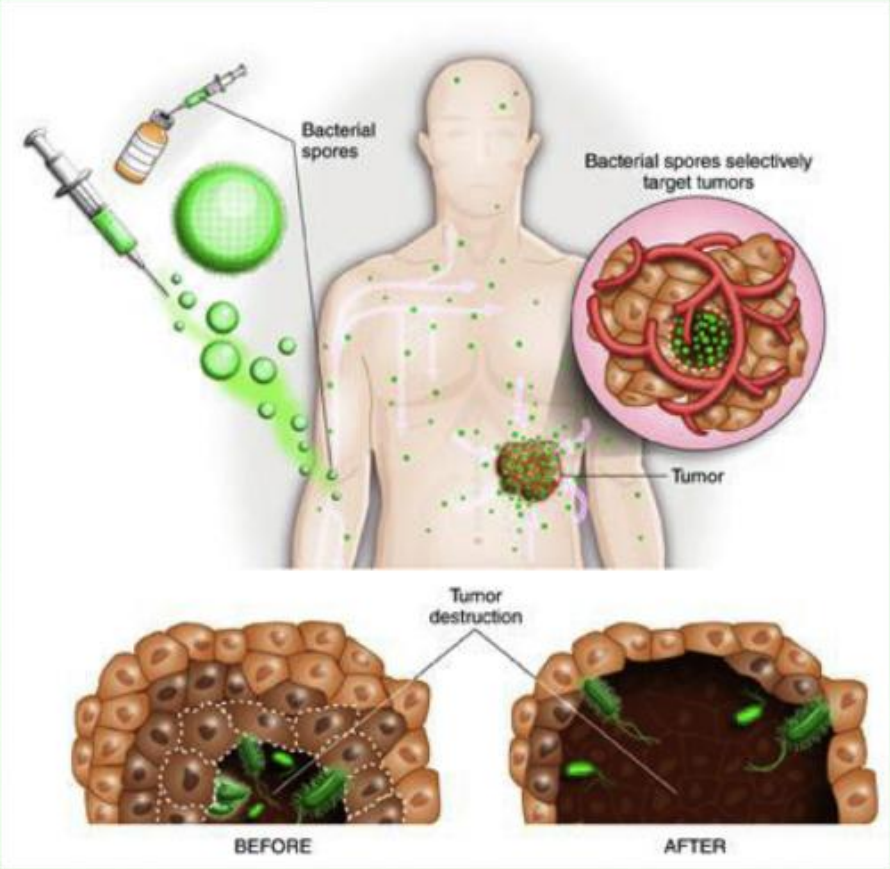
Bacteria as vector for gene therapy

- The specific targeting of therapy directly to a solid tumor
- The use of bacteria, **genetically engineered** to express a specific therapeutic gene
- Bacterial vectors can provide a powerful adjuvant therapy to various cancer treatments
- As vectors for delivering:
  - anticancer agents
  - cytotoxic peptides
  - therapeutic proteins to solid tumors

- A mutant of *S. typhimurium*, has been engineered to express interleukin-2 for the treatment of liver cancer in preclinical models

Bacterial spores

- The spores of ***anaerobic bacteria***
- The spores can germinate in the dead areas inside tumors
- Spores of genetically modified strain *C. novyi*
- No clinical toxicity was observed in healthy mice or rabbits



Bacterial toxins for cancer treatment

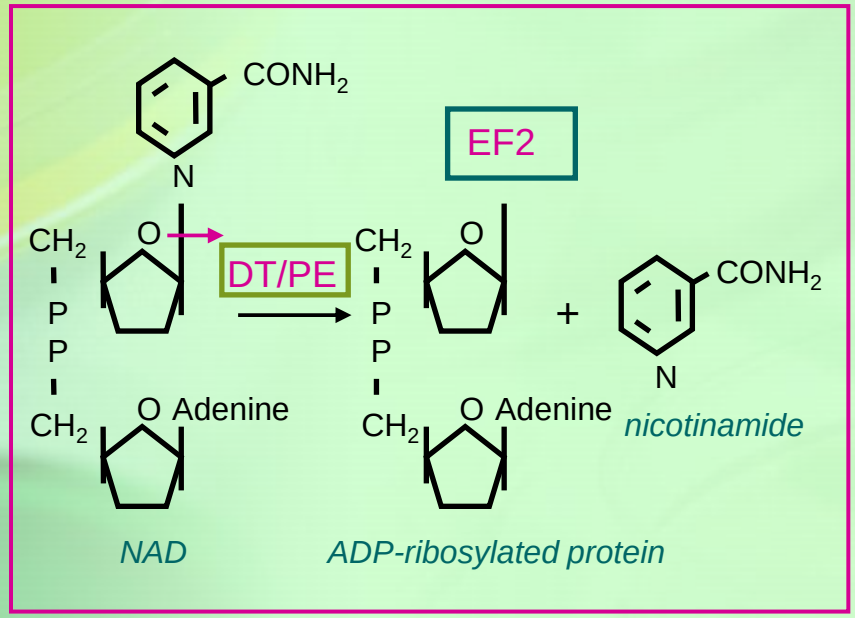
- ✓ Diphtheria toxin (DT)
- ✓ Pseudomonas exotoxin A (PE)
- ✓ Clostridium perfringes type A enterotoxin (CPE)
- ✓ **Anthrax toxin**

- ➢ The most potent cell-killing agents
  - ➢ **Need to be targeted** to specific sites on the surface of cancer cells

Bacterial toxins for cancer treatment

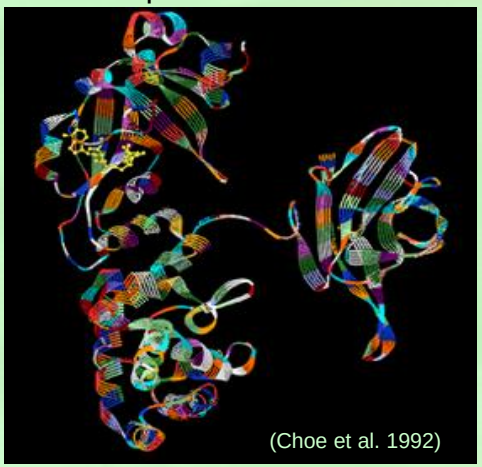
- ✓Diphtheria toxin (DT)
- ✓*Pseudomonas* exotoxin A (PE)

ADP-ribosyltransferase activity

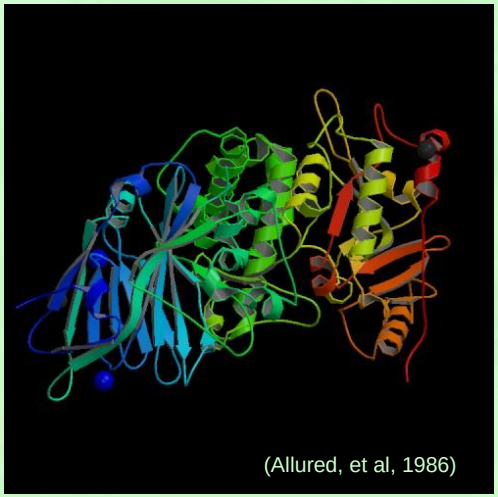


(Kabat, Iglewski, et al.)

Diphtheria toxin



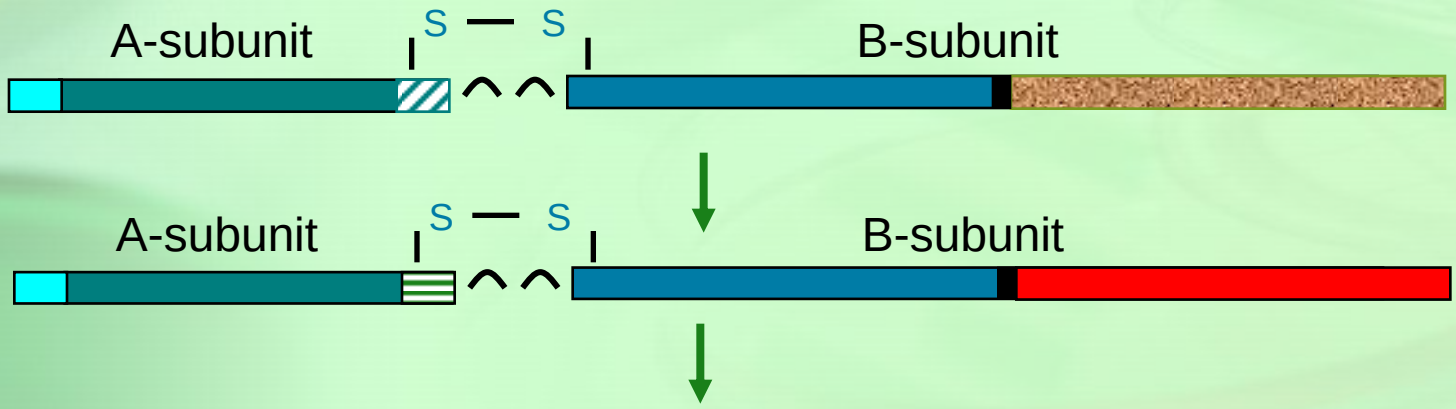
Pseudomonas exotoxin A



by: Mohammad Sekhavati /
Dr. M. Aslanimehr

Design of toxins as anti-cancer agents (immunotoxins)

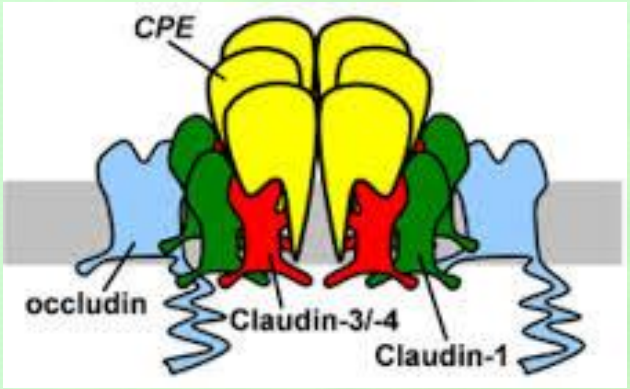
- A-B structure
  - B-domain: interaction with receptors
  - A-domain: toxic domain
- Recombinant immunotoxins: A toxic domain fused to
  - monoclonal antibodies
  - antibody fragments
  - ligands like cytokines and growth factors
- Clinical trials: performed with positive results in **leukemia** and **bladder cancer**



Bacterial toxins for cancer treatment

Clostridium perfringes type A enterotoxin (CPE)

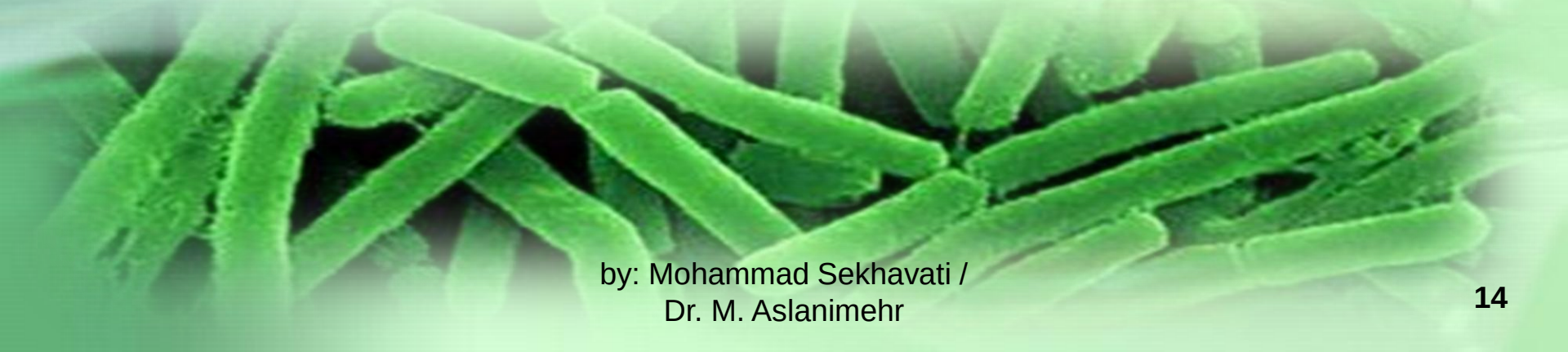
- The causative agent of gastroenteritis
  - The C terminal: binding to the CPE receptor (CPE-R)
  - The N-terminal: cytotoxicity
- Purified CPE: an acute cytotoxic effect on **pancreatic cancer** cells (tumor necrosis and inhibition of tumor growth *in vivo)*
- **Claudin-3 &4** are the specific receptors for CPE: are abundantly expressed in ovarian, breast, uterine, and pancreatic cancers



Bacterial toxins for cancer treatment

Anthrax toxin

- Harry Smith in 1954
- The major virulence factor of *Bacillus anthracis*
  - Aerobic, facultative anaerobic, spore-forming, nonmotile, nonhemolytic gram positive rods
  - Key virulence genes are found on plasmids **pXO1** and **pXO2**
  - pXO1: the structural genes for the anthrax toxin proteins
  - pXO2: carries three genes required for capsule synthesis (poly-D-glutamic acid)



Anthrax toxin

- Consists of three individually non-toxic proteins (**A-B** Toxin)
- Assemble at the mammalian cell surface into toxic complexes

Protective Antigen (PA)

- 83-kDa (63 , 20-kDa)
- Ability to elicit a protective immune response against anthrax
- Common cell binding domain (B)
- forms an oligomeric translocase channel
- delivers the two enzyme components

Edema Factor (EF)

- 89-kDa
- adenylate cyclase (calmodulin dependence) ↑cAMP
- EF plus PA creates edema toxin (ET)

Lethal Factor (LF)

- 90-kDa
- zinc-dependent→ protease cleaves MAPKKs → **apoptosis**
- LF plus PA creates lethal toxin (LT)

Pathogenesis

- ✓ Cutaneous – gastrointestinal - inhalation (deadliest form)

- The spores are taken up in **macrophages**
- Macrophages are then transported to the **lymph nodes**
- The spores enter the vegetative state in the macrophages
- The infected macrophages lyse and bacteria are released into the blood stream
- Virulence factors help the bacteria to evade the host's immune system
- Death from virulent *B. anthracis* is due to the production of a toxin that shuts down the host's immune system and causes cell death

Anthrax Toxin Mechanism

1. Binding of **PA** to a cell surface receptor
   - Receptors: TEM-8 (ANTXR1) and CMG-2 (ANTXR2)
2. Receptor-bound PA is cleaved into two fragments by a **furin family protease** (Dissociation of the smaller fragment)
3. The receptor-bound carboxyl-terminal 63-kDa fragment (PA63) to form a ring-shaped heptamer (lipid rafts)
4. The oligomerization of PA63 provides the binding site for LF and EF
5. The resulting complexes are endocytosed to an acidic compartment
6. In acidic endosomes, the complex forms pores through which lethal factor reaches the cytosol
7. EF or LF gets injected into the cytosol of the cell

Anthrax Toxin Mechanism

Edema Toxin

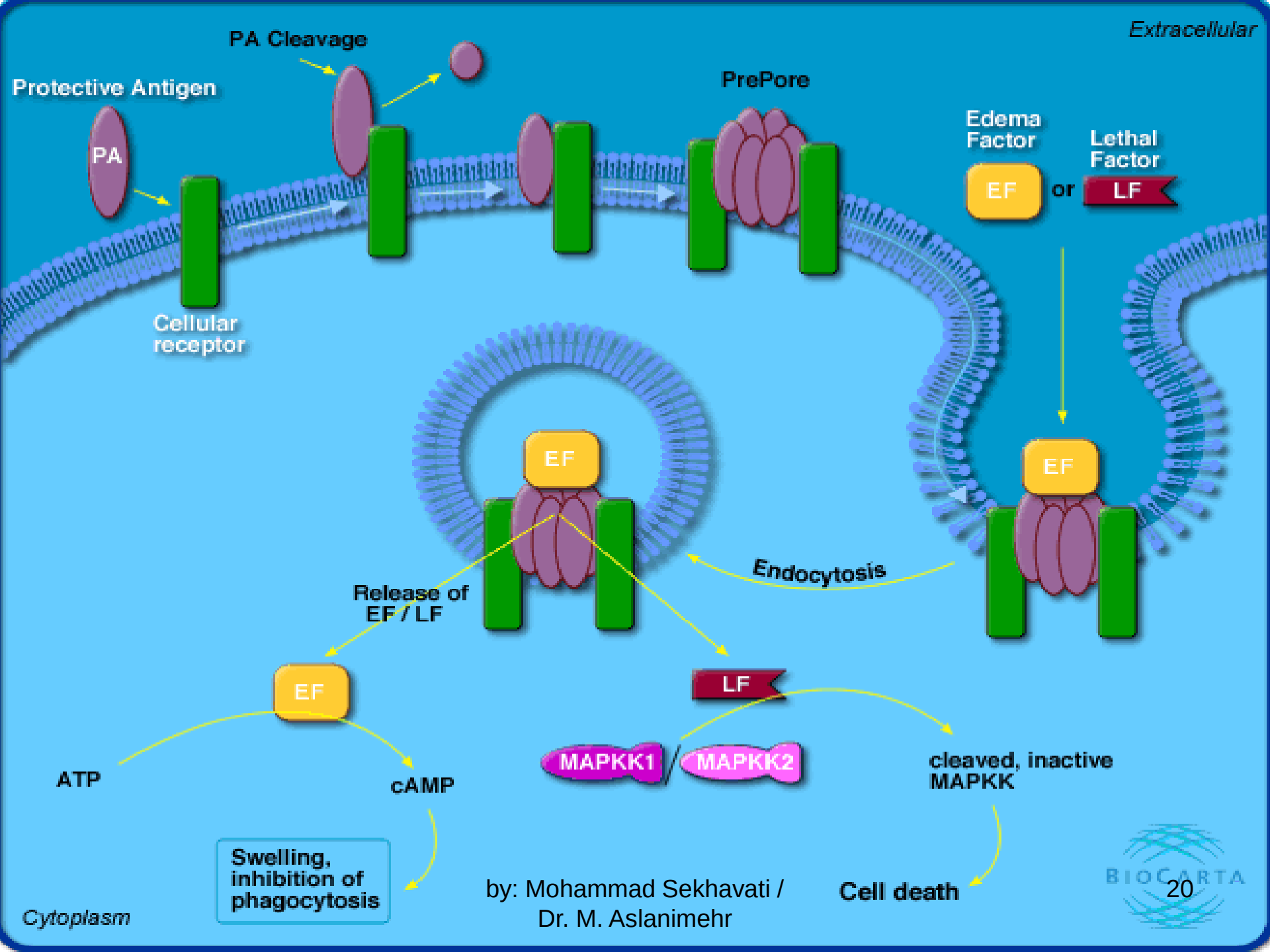
- A Ca^{2+}, calmodulin-dependent adenylate cyclase
- catalyzes the conversion of ATP to cyclic AMP (cAMP)

1. An increase in the amount of cAMP causes the cell to swell
   - cAMP is an important regulatory molecule (maintain proper osmotic pressure by regulating the flow of small ions and water in and out of the cell)
2. A decrease ATP (Macrophages need the energy/infected macrophages become useless)

Anthrax Toxin Mechanism

Lethal Toxin

- A zinc protease
- Targets members of the mitogen-activated protein kinase kinase (MAPKK) family
- Leads to inhibition of several cell signaling pathways
- An increased amount of cytokines, protein that act as cell mediators
- High cytokine levels cause an increase in harmful oxidative molecules. If the concentration of these harmful molecules gets too high within the cell, the macrophage ruptures and dies


Extracellular
PA Cleavage
Protective Antigen
PA
PrePore
Edema Factor
Lethal Factor
EF
or
LF
Cellular receptor
EF
EF
Endocytosis
Release of EF / LF
EF
LF
ATP
cAMP
MAPKK1
MAPKK2
cleaved, inactive MAPKK
Swelling, inhibition of phagocytosis
Cell death
Cytoplasm

Anti- Angiogenic And Tumoricidal effects of Anthrax Toxin

Prerequisite for invasive growth and metastatic spread of tumors:

1. Angiogenesis (The growth of new blood vessels)
   - Inhibition of angiogenesis is an important strategy for current anti-cancer therapies
- TEM-8 and CMG-2 (anthrax receptors) :
  - In **epithelia lining** of skin, lung, and small intestine (in normal)
  - overexpressed during angiogenesis
- Functional roles in angiogenesis:
- TEM-8: to regulate endothelial cell migration and **tubule formation**
- CMG-2: endothelial proliferation

CMG-2 and TEM-8 are potential targets for anti- angiogenic therapy

Anti- Angiogenic And Tumoricidal effects of Anthrax Toxin

- Anti-angiogenic effects of anthrax toxin:
  - LT (MAPK inhibition) and ET (↑ cAMP)
- The unique requirement of PA to make its activation
  - provides an opportunity to **re-engineer** this protein
- A number of mutations to PA increase its selectivity and targeting of tumor cells
  - replacing the furin activation site on a recombinant anthrax toxin with a Urokinase plasminogen activator (uPA) activation site.
- uPA are overexpressed in human tumors of:

  colon cancer, breast cancer, bladder cancer, thyroid cancer, liver cancer, pleura cancer, lung cancer, pancreas cancer, ovaries cancer, and the head and neck cancer

Anti- Angiogenic And Tumoricidal effects of Anthrax Toxin

2. Dissolution of the extracellular matrix
   - serine, metallo-, and cysteine proteases
- uPA:
  - serine protease
  - overexpressed in a variety of human cancers
- The active uPA/uPAR complex cleaves plasminogen into plasmin
- Absent on normal cells (wound healing)

Take advantages of

- The expression of the urokinase system on tumor
- The obligatory requirement for proteolytic processing of PA
- To target uPA-expressing tumor cells with modified anthrax toxin
  - The native furin cleavage site was replaced by a uPA cleavage

A urokinase-activated recombinant anthrax toxin is selectively cytotoxic to many human tumor cell types

Ralph
Shihu
and A
Ravibhushan Singh,[1]

To specifically target uPA

Furthermore, we identify three molecular markers, anthrax toxin receptor, uPA, and uPA receptor, which can be used
cell sensitivity to PrAgU2/FP59.

Materials and Methods

Toxins

PrAg, PrAgU2, and FP59 were made as described previously (11). PrAg and PrAgU2 have a molecular weight of 83 kDa, whereas the molecular weight of FP59 is 59 kDa. The purity of all three proteins used was >99%.

Cells and Cell Lines

All human cancer cell lines were purchased from the American Tissue Culture Collection (Manassas, VA) and cultured as recommended. Normal human cells were purchased from Cambrex (Baltimore, MD) or American Tissue Culture Collection and were cultured as recommended.

Cytotoxicity Assay

Cytotoxicity was determined using a [^{3}H]thymidine incorporation inhibition assay as described previously

Related Studies

- In 1976: Discovery that uPA is produced and released from cancer cells (roles in the metastasis of human tumors)
- Serve as targets for novel cancer
- Interfering with uPA (antibodies, inhibitors, and synthetic uPA analogues)
- Only the progression of tumors get slow without a cytotoxic action

- In 2001, Liu: to exploit the protease activity of uPA to target cytotoxic bacterial toxin fusion proteins to tumor cells
- PA/FP59: mutated anthrax toxin-protective antigen fused to the Pseudomonas exotoxin A (FP59)
- Treatment of tumor cells (HeLa cells, human melanoma A2058 cells) with PA/FP59

Thanks for your attention

by: Mohammad Sekhavati /
Dr. M. Aslanimehr

The man who makes no

mistake,

makes nothing